



Letter to the Editor

THAP1 mutations in a Greek primary blepharospasm series

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Benign essential blepharospasm (BEB) is an adult onset focal dystonia that manifests as forced involuntary eyelid closure usually starting between the fifth and the seventh decade [1]. The frequency is rare; affecting around 1 in 20,000 people, where most of the cases are sporadic, approximately 20–30% of patients may have a positive family history, thus indicating a possible genetic contribution in the aetiology of blepharospasm [1].

A recent study examined the genetic influences in blepharospasm based on the examination of patients and their first-degree relatives and found a very high proportion of probands (27%) with at least one first-degree relative affected by some form of focal dystonia [1]. Polymorphisms of the genes encoding *TOR1A* and *D5* dopamine receptor (*DRD5*) have been associated with an increased lifetime risk of focal dystonia and these genes were investigated in two independent cohorts of Italian and North American patients with blepharospasm [2]. While no association was identified, analysis of the Italian group separately revealed an association with the same *TOR1A* risk haplotype as previously described in an Icelandic population.

Over the last decade the number of dystonia loci and disease genes have grown dramatically [3]. The focal dystonia group consists of *DYT1*, *DYT4*, *DYT6* and *DYT7* but only three genes have been recognised up to now, *TOR1A* in *DYT1*, *THAP1* in *DYT6* and *CIZ1* recently.

THAP1 mutations are associated with a number of dystonia phenotypes, variability in severity and progression. In the dystonia cases that have been analysed so far, a handful of dystonia cases have been identified where blepharospasm was part of an overall segmental or generalised dystonia phenotype but no cases with BEB had been reported. Based on the reported data we investigated the role of *THAP1* gene in blepharospasm in a Greek patient series.

All patients and healthy controls that participated in the study gave informed consent and the study was approved by the local ethics committee of the University Hospital in Larissa, Greece. The diagnosis of blepharospasm was based on accepted criteria. Secondary causes such as structural lesions, trauma or exposure to toxic or neuroleptic substances were excluded. All patients

were negative for the GAG deletion in the *TOR1A* gene. A total of 70 patients of Greek ancestry were included in this genetic screening study (Table 1). 200 healthy controls were also screened for the *THAP1* gene. Most patients were regularly receiving botulinum toxin.

Standard PCR amplification was carried out as previously described. All patients were screened for mutations in *THAP1* by sequencing.

Prediction programs PolyPhen (<http://genetics.bwh.harvard.edu/pph/>), Align-GVGD (<http://agvgd.iarc.fr/>) and SIFT (<http://sift.jcvi.org>), were used to examine possible effects of identified mutations and polymorphisms (RefSeq NM_018105.2). Human Splicing Finder tool was used to evaluate mutations that potentially affect splicing (<http://www.umd.be/HSF/>).

In the 70 patients screened, 2 patients were found to carry heterozygous *THAP1* variations. The patient with the c.208 A>G (K70E) variation was a 64-year old woman who had blepharospasm since the age of 55 years. Family history was negative for dystonia. This mutation is a missense amino acid change, it is present in the *THAP1* domain and was not seen in Greek controls and has not been reported in any control series published or available on-line.

The second patient had a heterozygous *THAP1* intronic variation c.-40T>C was a 75 year old woman who first was diagnosed with blepharospasm at the age of 64. However, 2 years later she developed oromandibular symptoms with spasms of jaw opening. A diagnosis of segmental craniocervical dystonia was made at that point. She didn't report positive family history for dystonia. The variation is located in the promoter region 40 bases before the first exon start and therefore it is possible to be implicated in the regulation of gene expression. Both patients were working as farmers and they both reported exposure to pesticides since they were young, for over 30 years.

We sequenced the *THAP1* gene in a cohort of Greek patients with blepharospasm identifying one likely pathogenic mutation and one likely benign intronic variant. The heterozygous missense K70E mutation that we identified is located within the *THAP1*

Table 1

Clinical details of the patients with benign essential blepharospasm and healthy controls screened for *THAP1* mutations.

Patients (total number)	70
Mean age (range)	66 (49–85)
Mean age at onset (range)	59 (30–79)
Gender (females)	74%
Non-smokers	86%
Exposure to antipsychotic agents	0%
Controls (total number)	200
Mean age (range)	68 (42–91)
Gender (females)	67%

domain, next to a number of other likely pathogenic *THAP1* mutations, these were all absent in controls. The 5'UTR mutation c.-40T>C reported here may contribute to blepharospasm by affecting the secondary structure of mRNA or through other post-transcriptional regulation. This variant is in close proximity to the c.-42C>T variation that has been reported in two unrelated Caucasian women with focal dystonia, one with blepharospasm and the other one with laryngeal dystonia [4]. Both patients were women without positive family history of dystonia, whereas age of disease onset was 62 and 69 years respectively which is quite similar to the age of onset of our patient (64 years). Although UTR and intronic mutations warrant discussion, seem to be frequently associated with the dystonia phenotype and are not present in controls. Disease-associated variants in the 5'UTR of human protein coding genes have been systematically analysed and functionality of these variants correlated highly to predicted secondary structural changes. However, there is still little evidence to suggest that these variants alter the expression or structure of *THAP1* gene in dystonia patients.

Prior to this study some other patients with blepharospasm were screened for *THAP1* mutations and the two intronic changes discussed above were identified [4]. In addition two cases with blepharospasm as part of a more complex dystonia phenotype with non-synonymous *THAP1* mutations were reported. Given the small number of cases screened further analysis of the *THAP1* gene is required. As well as *THAP1* mutations, blepharospasm can be part of other genetic phenotypes such as Mohr-Tranebjaerg syndrome [5], in general these associations are rare and the aetiology of this type of cranial dystonia remains largely unknown.

Our findings indicate that *THAP1* mutations are very rare in this condition but with the advent of rapid genetic screening should be considered in patients, particularly if there is a family history. Further genotype phenotype correlations in *THAP1* will be important combined with investigations into the functional consequences of these mutations.

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None.

Conflict of interest

None.

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